

**DETERMINATION OF PROBIOTIC PROPERTIES OF LACTIC ACID BACTERIA
FROM KAZAKH DAIRY PRODUCTS****K.Zh. Tleuova¹, A.U. Shingisov², S.S. Vetokhin³**^{1,2}NJSC “South Kazakhstan University named after M. Auezov, Kazakhstan 160012, Shymkent,
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Abstract. *The research was carried out in the laboratory of the Research and Production Center of Virology and Microbiology of the Republic of Kazakhstan and at the Institute of Microbiology of the Academy of Sciences of the Republic of Uzbekistan, laboratory of Microbiology and biotechnology of probiotics. The object of the study were newly isolated strains of lactobacilli. During the selection work, generally accepted and standard methods of microbiological analysis were used. The morphological-cultural, physiological-biochemical and technologically valuable properties of 12 strains of lactic acid bacteria isolated from objects of natural origin were studied. The objects for isolating lactic acid bacteria were natural cow's, goat's, mare's, and raw camel's milk. Identification of the isolated strains of lactic acid bacteria was carried out according to the key properties specified in the ninth edition of the Bergey Guide. Based on the results of the studies, the newly isolated strains of lactic acid bacteria were classified as *Lactococcus lactis* ssp. Passports have been compiled for the isolated strains of lactococci. The newly isolated strains of lactic acid bacteria were included in the Republican State Enterprise “Republican Collection of Microorganisms” of the Committee of Science of the Ministry of Education and Science of the Republic of Kazakhstan (RKM) as promising strains for inclusion in the composition of bacterial starters in the production of fermented dairy products.*

Keywords: *lactic acid bacteria, selection of lactococci, physiological and biochemical properties, organoleptic characteristics, technologically valuable properties, thermal resistance, salt tolerance, phage sensitivity, antibiotic resistance, fermented dairy products. Kazakhstan, a country with rich natural resources and a variety of agricultural products, is one of the leaders in the production of dairy products. Research shows that Kazakh dairy products such as milk, kefir, yogurt and cottage cheese contain high levels of lactic acid bacteria such as *Lactobacillus* and *Bifidobacterium*. These bacteria are part of the normal microflora of the digestive system and can have a positive effect on our health.*

The goal is to determine the probiotic properties of lactic acid bacteria isolated from Kazakh dairy products. Interest in this topic is justified by the significant potential for the use of lactic acid bacteria in functional products, as well as the need to study their probiotic properties for the development of the food industry in Kazakhstan.

Sourdough is an important component of the quality of fermented milk products. Various strains and types of lactic acid bacteria (lactococci, thermophilic streptococci, lactobacilli), bifidobacteria, and propionic acid bacteria are used as starter microflora. Lactic acid bacteria convert the main components of milk (lactose, protein and fat) into taste, aromatic, biologically active substances and suppress the growth of technologically harmful microorganisms. The process of obtaining starter cultures is labor-intensive and includes the following main stages of work: isolation of microorganisms from various sources, their study, selection of promising strains

with properties valuable for production, and subsequent selection of pure cultures for the preparation of bacterial starter cultures. Isolation of pure cultures of lactic acid bacteria is the basis for creating and maintaining industrial collections of microorganisms. Various foreign and domestic research institutes are engaged in the search for competitive cultures of lactic acid bacteria with the subsequent development and production of starter cultures and enriched bacteria [1, 2].

Lactic acid bacteria, which are part of starter cultures and concentrates of lactic acid bacteria, make it possible to obtain fermented milk products with different taste characteristics characteristic of certain types of products. Most starter cultures and concentrates of lactic acid bacteria used in the production of cheese, kefir, sour cream, cottage cheese and butter contain mesophilic lactobacilli.

When developing new bacterial starter cultures and concentrates, multi-stage work is carried out to select lactic acid bacteria with valuable properties. Most lactic acid bacteria isolated from natural sources often do not meet the requirements for starter strains used in the dairy industry. In this case, inactive ones must be replaced with new starter microorganisms.

Since, under production conditions, starter microflora is subject to lysis by bacteriophages, when selecting starter microorganisms, breeders take into account the phage resistance of strains. The activity of lactic acid microflora is one of the main conditions for obtaining high-quality fermented milk products, both in terms of histological indicators and safety indicators; 75-95% of the reasons for the loss of starter activity and poor-quality production of fermented milk products are associated with the destruction of the starter microflora by bacteriophages. Phagocytosis of starter microflora leads to a stop of the lactic fermentation process and the resulting processes may not meet quality indicators and be unsafe for consumption [3, 4, 5].

Collections of microorganisms in industry must have a certain set of strains with identical characteristics for the preparation of bacterial compositions, which are subsequently used in the rotation of starter bacteria in production.

Despite the existence of collections of strains of lactic acid bacteria actively used for industrial purposes, the search for new strains of mesophilic lactic acid bacteria to obtain fermented milk products with specific properties and replenishment of the collection with newly isolated strains with technically valuable characteristics does not lose its importance [6, 7, 8]. To maintain and replenish the collection, it is necessary to constantly work on isolating promising strains of lactic acid bacteria, which can later be used in the production of bacterial starter cultures and bacterial concentrates for the production of fermented milk products [9, 10].

The objective of the ongoing research is to isolate promising strains of lactococci in bacterial starter cultures used in the production of fermented milk products. The selected promising strains will be included in the Republican State Enterprise "Republican Collection of Microorganisms" of the Scientific Committee of the Ministry of Education and Science of the Republic of Kazakhstan.

Isolates of lactic acid bacteria were obtained from natural raw cow, mare, goat, and camel milk.

Isolation of pure cultures of lactococci was carried out by repeated passage in sterile skim milk, followed by incubation at the optimal temperature for aerobic lactococci (30 ± 1 °C). Isolates were identified by morphological, cultural and physiological-biochemical characteristics of the Bergey Determinant [11].

Isolation of mesophilic lactococci includes the following steps: sampling; isolation of pure cultures; research of morphological, cultural and physiological-biochemical characteristics to identify isolated strains, testing of strains for technically valuable characteristics; creation of strain passports; inclusion of promising strains of mesophilic lactococci in RCM.

Standard and generally accepted methods of microbiological analysis were used [12, 13, 14].

A total of 96 strains were isolated. The isolated cultures formed a clot in sterile skim milk within 24 hours when inoculated with one bacteriological loop, while the ability to form a uniform, dense clot, morphological characteristics (appearance of colonies, microscopic specimen), physiological and biochemical characteristics (Gram stain, activity catalase, ammonia production from arginine, absorption of calcium citrate carbohydrates).

Based on the results of these studies, 12 strains were selected for further study and assigned to the species *Lactococcus lactis* ssp., based on the main properties of Bergey's definition. Selected 12 strains of *Lactococcus lactis* were studied for such technically valuable properties as acid-producing activity, organoleptic properties, gas and aroma-forming activity, salt resistance, heat resistance, antibiotic resistance, and phage resistance.

One of the important indicators of lactic acid bacteria is acid-forming activity, which characterizes the fermentation of lactose. This indicator for the tested strains was assessed by titratable acidity after 4 hours of incubation and the time of clot formation at 5% of the culture inoculum. Most strains showed good acid-forming activity. Acidity after 4 hours of incubation varied within 44-61 oT for 10 strains and 33-42 oT for 2 strains. All strains (12) formed a clot within 6-8 hours, and titratable acidity varied within 71-100 oT.

Histological characteristics of lactic acid bacteria are important as selection criteria when preparing bacterial starter cultures. According to the tasting results, the isolates were characterized by dense coagulation, homogeneous consistency and pure fermented milk taste.

According to the gas-forming ability (gas-forming activity) of ideacyl-acetoin producing ability (flavor-forming activity) of their 12 strain isolates, two showed gas-forming ability (gas-forming activity).

Of the 12 isolates, two strains showed positive results for aromatic and gas-forming activity.

The tolerance of lactococci to various concentrations of table salt (% NaCl) is most relevant for cheese production. Salt controls microbiological, biochemical and physical-chemical processes during the production and ripening of cheese, influencing the formation of taste and consistency. Therefore, lactic acid bacteria included in bacterial starter cultures must be tested for resistance to sodium chloride concentrations (% NaCl).

The studied strains showed growth on nutrient media. Containing 2% and 4% NaCl; a concentration of 6.5% NaCl had an inhibitory effect on the growth of the studied strains.

The ability of microorganisms to withstand high temperatures at one time plays an important role in the production of cheese during reheating. Studies have shown that all isolated lactococci are able to withstand heating at 65°C for 30 and 60 minutes.

Milk coming from dairy processing plants often contains inhibitors, including antibiotics. Technical Regulations of the Customs Union TR CU 033/2013 "On the safety of milk and dairy products" establishes acceptable levels of antibiotics in milk and dairy products [15, 16]. Most antibiotics suppress the growth of lactic acid starter flora. The susceptibility of lactic acid

bacteria to antibiotics varies. Therefore, antibiotic resistance should be taken into account when selecting pure culture strains for starter cultures.

Isolated strains. Tested for resistance to antibiotics such as gentamicin, tetracycline and azithromycin.

The results showed that 10 lactococci were resistant to gentimycin at a concentration of 0.005 mg/l, two were weakly resistant; eight were resistant to tetracycline, at a concentration of 0.01 mg/l, 4 were weakly resistant; seven were resistant to azithromycin, at a concentration of 0.2 mg /l, the remaining 5 strains were weakly resistant.

Sensitivity to phages is taken into account when selecting lactic acid bacteria. Resistance of isolates to bacteriophages was determined by co-cultivating the test strains with bacteriophages in hydrolyzed broth. The results were assessed by the presence or absence of growth of the studied crops.

To assess the degree of sensitivity of strains to phages, the sensitivity index (SSI) was used. It is the ratio of the number of phages that lyse the strain to the total number of phages used [17]. In accordance with the phage sensitivity index, lactococcal isolates were classified as follows: 8 weakly sensitive (IF0. 3), 4 are moderately sensitive (0.31-0.3), (CN 0.31-0.7)

Based on the results of the study, a passport was compiled for 12 lactococcal isolates, characterized by morphological and cultural, physiological-biochemical and technically valuable indicators. These strains are included in the Republican State Enterprise “Republican Collection of Microorganisms” and are maintained by transfer to nutrient media.

However, isolated microorganisms may lose their technically valuable properties when stored in subcultures. Therefore, to maintain the collection, it is necessary to continue work on isolating lactobacilli strains

Isolated *Lactobacillus* strains must be subjected to genetic analysis for species identity, as they can be included in the bacterial starter culture.

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